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Cladophialophora pucciniophila, a new hyphomycete parasitizing a rust fungus

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Abstract— The hyperparasitic hyphomycete *Cladophialophora pucciniophila* sp. nov., collected on rust telia of *Puccinia polygoni-amphibii* from Korea, is described, illustrated, and compared with allied species. The new species differs from previously described *Cladophialophora* species in having allantoid conidia and hyperparasitism on a rust fungus. Phylogenetic analysis of this fungus based on ITS rDNA data confirms its placement within the genus *Cladophialophora*.

Key words— anamorphic fungi, *Capronia*, fungicolous species, mycoparasitism

Introduction

The dematiaceous hyphomycete genus *Cladophialophora* was established by Borelli (1980) with the type species *C. ajelloi*, now regarded as synonymous with *C. carrionii* (Trejos) de Hoog et al. (Badali et al. 2008). The anamorph genus *Cladophialophora*, which has a close affinity to the teleomorph genus *Capronia* Sacc., is phylogenetically placed in the family *Herpotrichiellaceae* (order *Chaetothyriales*) (Haase et al. 1999, Untereiner 2000). Members of *Cladophialophora* produce simple conidiophores, often reduced to conidiogenous cells, with catenate conidia formed in branched or unbranched acropetal chains. The genus currently comprises fewer than 30 species, which are opportunistic human pathogens and phytopathogens or are isolated from environmental sources (Crous et al. 2007, Davey & Currah 2007, Badali et al. 2008, 2009, 2010, Koukol 2010).

During our extensive investigations on the phytopathogenic fungi in Korea, rust telia hyperparasitized by a hyphomycetous fungus were found on leaves of *Persicaria fauriei*. Based on morphological characteristics and molecular analysis, the hyperparasitic fungus represents an undescribed species belonging

to the genus *Cladophialophora*. In this study, the fungus is described and illustrated as a novel species.

Materials & methods

Fresh and air-dried specimens were used for morphological observations. For microscopy, free hand sections of fungal structures were mounted in water and 3% KOH solution. Taxonomic characters were measured at magnifications of 200× and 400× using an eye-piece micrometer and a model BX51 microscope (Olympus, Tokyo, Japan). Microscopic photographs were taken using an Axio imager microscope (Carl Zeiss, Göttingen, Germany). A voucher specimen has been deposited in the Korea University herbarium (KUS-F23645).

A monoconidial isolate was grown on potato dextrose agar (PDA) at 25°C in the dark and deposited in the Korean Agricultural Culture Collection of the National Academy of Agricultural Science, Korea (KACC43957). Harvested mycelia were used for genomic DNA extraction following a previously described method (Lee & Taylor 1990). ITS and 28S rDNA regions were amplified using primers ITS1/ITS4 and LROR/LR7, respectively (White et al. 1990). The obtained PCR products were purified using a LaboPass PCR purification kit (Cosmo Genetech, Seoul, Korea) and were directly sequenced using an ABI Prism™ 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA) using a BigDye™ cycle sequencing kit version 3.1 (Applied Biosystems) with the same primer pairs used for PCR. The raw sequences were edited using the DNASTAR computer package version 5.05 (Lasergene, Madison, WI, USA). The ITS and 28S sequences obtained have been deposited in GenBank with the accession numbers JF263533 and JF263534, respectively. For phylogenetic analysis, all available ITS rDNA sequences of *Cladophialophora* spp. were retrieved from GenBank. A phylogenetic tree was constructed by the neighbor-joining method and the general time reversible model (GTR + G) using PAUP* version 4.0b10 (Swofford 2002), and confidence levels for the individual branches of the tree were estimated with 1000 bootstrap replications. In this study, however, the phylogenetic analysis based on 28S rDNA sequences was not conducted to infer the relationship among *Cladophialophora* spp. because of the insufficient number of taxa available in GenBank.

Taxonomy

Cladophialophora pucciniophila M.J. Park & H.D. Shin, **sp. nov.** FIGS. 1–2

MycoBank MB 561051

Caespituli plerumque in teliis, punctiformes vel effusi, olivaceo-virides vel cinerei. Mycelium primarium immersum; hyphae hyalinae, septatae, 1–2.5 µm latae. Stromata substomatalia, bene evoluta, prope telia hyperparasitice affecta, usque ad 50 µm diam., pallide flavo-brunnea. Mycelium secundarium externum; hyphae superficiales, septatae, ramosae, hyalinae, laeves, 1–4 µm latae. Conidiophora laxe vel modice densa, pauca vel modice numerosa, fasciculata, per stomata emergentia, ex hyphis repentibus oriunda, macronemata, micronemata, interdum in cellulis conidiogenis reducta, erecta vel decumbentia, recta, simplicia, interdum ramosa, subcylindrica vel cylindrica, 1–9-septata, pallide olivacea, laevia, 40–225 × 2.5–3.5 µm. Cellulae conidiogenae in conidiophoris incorporatae, terminales et intercalares, 5–15 µm longae, sympodialiter proliferantes,

subcylindricae; loci conidiogeni subtruncati vel truncati, vel conspicui, leniter incrassati et fuscati. Conidia ramosa vel non-ramosa catenata usque ad 10, recta vel leviter curvata, subcylindrica vel ellipsoidea, vel allantoidea, 0–1(–3)-septata, 20–38(–42) × 4–5 µm, pallide olivacea, laevia; hila subtruncata, vel conspicua, leniter incrassata et fuscata; ramoconidia plerumque praesentia. Coloniae in agaro PDA post 30 dies ad 25 °C 20–25 mm diam. attingentes, olivaceo-virides vel griseae, velutinae, ex mycelio aerio denso constantes; reversum olivaceo-nigrum. Teleomorphosis ignota.

Type: On rust telia of *Puccinia polygoni-amphibii* Pers. on *Persicaria fauriei* (H. Lév. & Vaniot) Nakai (*Polygonaceae*), Korea, Chuncheon, Bongmyeong-ri, 37°46′49″N, 127°48′55″E, 270 m a.s.l., 4 Sep. 2008, M.J. Park & H.D. Shin (KUS-F23645 **Holotype**; HAL 2431 F **Isotype**).

Etymology: The epithet refers to the genus of the mycohost.

Caespituli mostly confined to telia of *Puccinia polygoni-amphibii*, punctiform to effuse, olivaceous-green to ash-gray. Primary mycelium immersed; hyphae hyaline, septate, 1–2.5 µm wide, occasionally forming well-developed substomatal stromata in the vicinity of hyperparasitized telia, up to 50 µm diam., pale yellowish brown. Secondary mycelium external; hyphae superficial, septate, branched, hyaline, smooth, 1–4 µm wide. Conidiophores in loose to moderately dense, small to moderately large fascicles, emerging through stomata, or arising from superficial hyphae, macronematous, micronematous, sometimes reduced to conidiogenous cells, erect to decumbent, straight, simple, sometimes branched, subcylindrical to cylindrical, 1–9-septate, pale olivaceous, smooth, 40–225 × 2.5–3.5 µm. Conidiogenous cells integrated, terminal and intercalary, 5–15 µm long, proliferating sympodially, subcylindrical; conidiogenous loci subtruncate to truncate, or conspicuous, slightly thickened and darkened. Conidia in branched or unbranched chains of up to 10, straight or slightly curved, subcylindrical to ellipsoid, or allantoid, 0–1(–3)-septate, 20–38(–42) × 4–5 µm, pale olivaceous, smooth; hila subtruncate, or conspicuous, slightly thickened and darkened; ramoconidia frequently present. Colonies on PDA attaining 20–25 mm diam after 30 d at 25°C in the dark, olivaceous-green to gray, velvety, consisting of dense aerial mycelium, with entire margin; reverse olivaceous-black; sporulation not observed. Teleomorph unknown.

Comments — The newly isolated fungus was morphologically characterized by melanized simple conidiophores and coherent conidial chains. Thus, it was considered as a species of *Cladophialophora*, being consistent with the current genus concept (Borelli 1980, Ho et al. 1999). However, the presently reported fungus did not match well with descriptions of the known *Cladophialophora* species, indicating the probable new taxon. To confirm *C. pucciniophila* as novel, ITS rDNA sequence analyses of *C. pucciniophila* and other *Cladophialophora* species implied the phylogenetic position of *C. pucciniophila* within the genus. Previous phylogenetic studies (Crous et al. 2007, Badali et al. 2008) suggest that the plant-associated *Cladophialophora* species have distinctly evolved

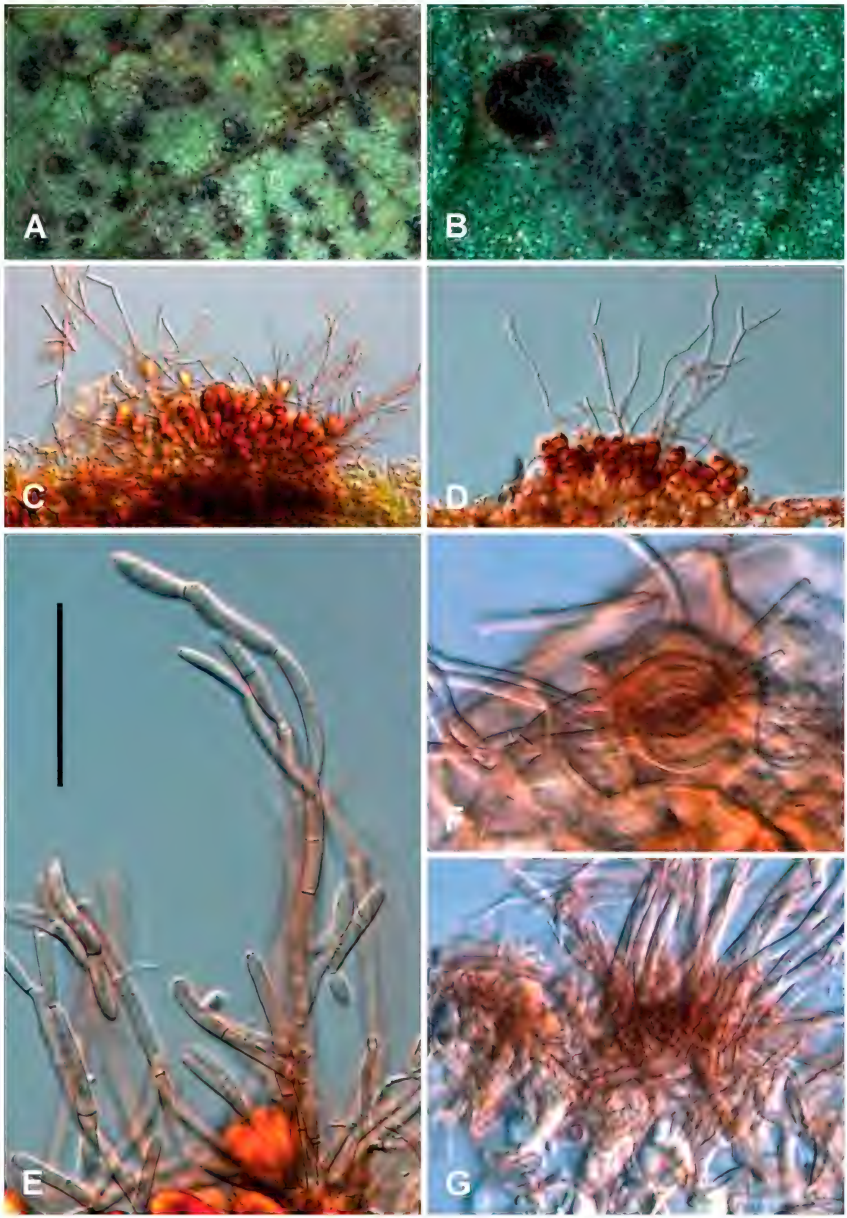

Fig. 1. Appearance of *Cladophialophora pucciniophila*. A–B. Colonies overgrowing on telia. C–D. Vertical sections of hyperparasitized telia. E. Conidiophores effused from rust telia. F–G. Substomatal stromata. Scale bars: C–D = 200 μm, E–G = 50 μm.

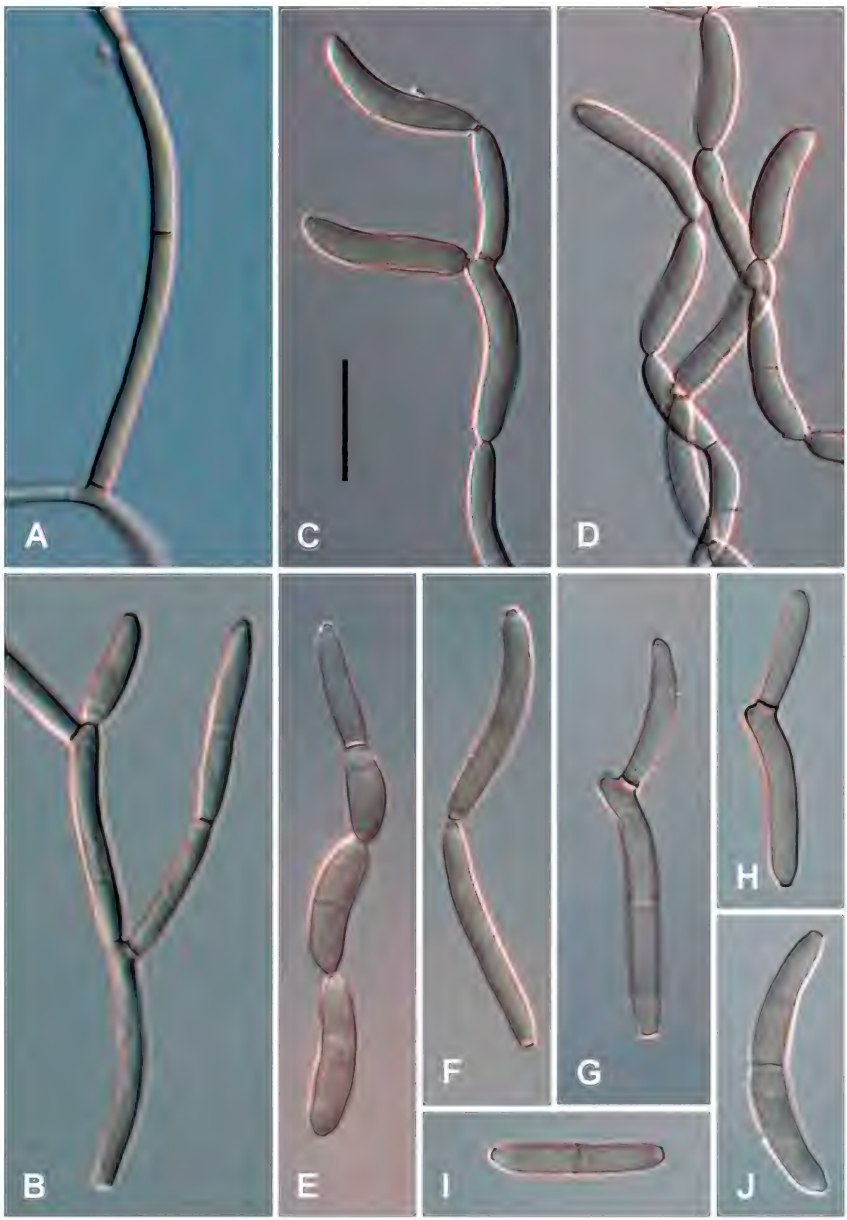

Fig. 2. Conidiophores and conidia of *Cladophialophora pucciniophila*. A. Basal part of conidiophore. B. Upper part of branched conidiophore bearing immature conidia. C–F. Catenate conidia. G–H. Ramoconidia. I–J. Conidia. Scale bar = 20 μm.

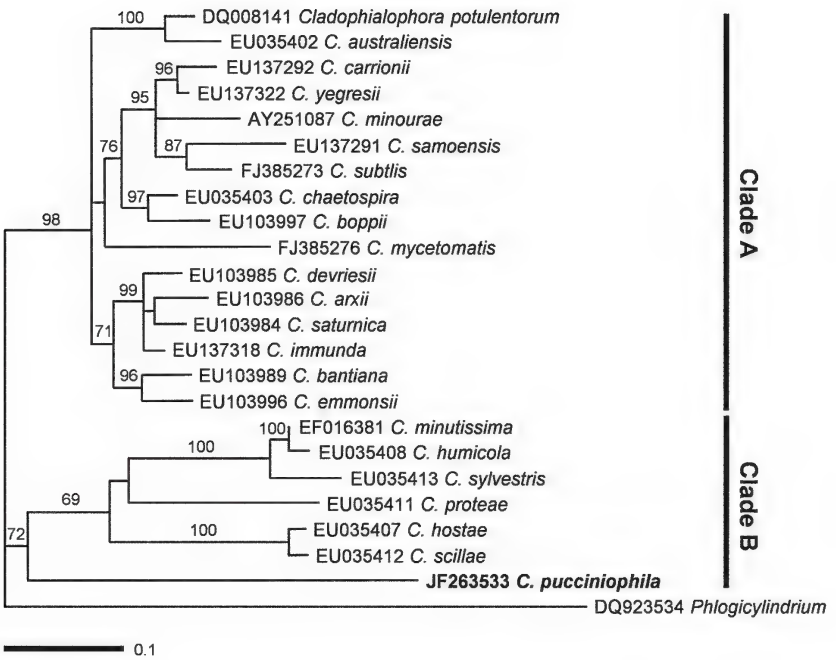

FIG. 3. Neighbor-joining tree showing the phylogenetic relationship among ITS rDNA sequences of *Cladophialophora* species. Numbers on the branches represent bootstrap values greater than 50% based on 1000 replications.

from saprobic and human-pathogenic species. The neighbor-joining tree (FIG. 3) largely clusters the *Cladophialophora* species into two clades, Clade A and Clade B, mainly according to the substrates. Clade A accommodated most *Cladophialophora* species that are human pathogens or environmental in origin. The remaining *Cladophialophora* taxa formed Clade B. Sequence analyses placed the new fungus, *C. pucciniophila*, as sister to a group of six *Cladophialophora* species within Clade B with moderate bootstrap value support (72%). Notably, all species in Clade B except *C. humicola* inhabit plant leaves, although the new species is a mycoparasite on a leaf-inhabiting rust.

C. pucciniophila may have diverged evolutionarily from the phytopathogenic species by acquiring a hyperparasitic ability as well as exploiting its new ecological niche on living plants. In this respect, it is noteworthy that *C. pucciniophila* forms small substomatal stromatic hyphal aggregations after penetrating into plant tissues via rust telia. Among the species phylogenetically

related to *C. pucciniophila*, *C. scillae* (Deighton) Crous et al. closely resembles the new taxon in conidial shape and septation. However, *C. scillae* differs from *C. pucciniophila* by having smaller conidia (10–20 × 1.5–3 μm) and longer conidial chains (≤ 30 in number; Crous et al. 2007). Although the two species produce similar subcylindrical to ellipsoid conidia, *C. pucciniophila* is distinctive in having allantoid conidia.

Recently, Badali et al. (2008) investigated the fungal biodiversity of *Cladophialophora*. Ecologically, *Cladophialophora* species are mainly human pathogens, plant pathogens, and environmental saprobes; fungicolous species have not previously been described in the genus. In this regard, it is intriguing for a member of *Cladophialophora* to be isolated from a rust fungus as a hyperparasite in the natural environment. The discrepancy in ecological habitats between *C. pucciniophila* and the other *Cladophialophora* species is additional evidence for recognizing the hyperparasitic fungus as a new species in the genus.

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